All work done according to standard protocols unless otherwise indicated

September, 2014

Monday, 1st

- Plates grew, picked and grown overnight in the appropriate two antibiotics

Tuesday, 2nd

- Culture miniprepped, cut with e and s to see if both plasmids were there, they were not, picked other colonies

Wednesday, 3rd

- Miniprepped, not both. Cotransformation done again. Rehydrated K608351 heat sensitive promoter and transformed. Prespun media for exosomes collection

Thursday, 4th

- Picked K608351 plates. K145151 rehydrated from kit plate and transformed. Picked cotransformation plates

Friday, 5th

- K608351 cultures prepped and confirmed. Picked k145151 plates. Cotransformation cultures grew, miniprepped and digested again and only one plasmid again.

Saturday, 6th

- RTH mutagenesis of S32-RBS to add universal adaptor (RFC25 suffix). Picked K145151 colonies and grew overnight

Sunday, 7th

- RTH mutagenesis transformed, miniprep of K145151 and digest. Size is good. Miniprepped K145151

Monday, 8th

- S32-B0034 universal mutagenesis plates picked.

Tuesday, 9th

- S32-B0034-Uni cultures miniprepped and sequenced

Wednesday, 10th

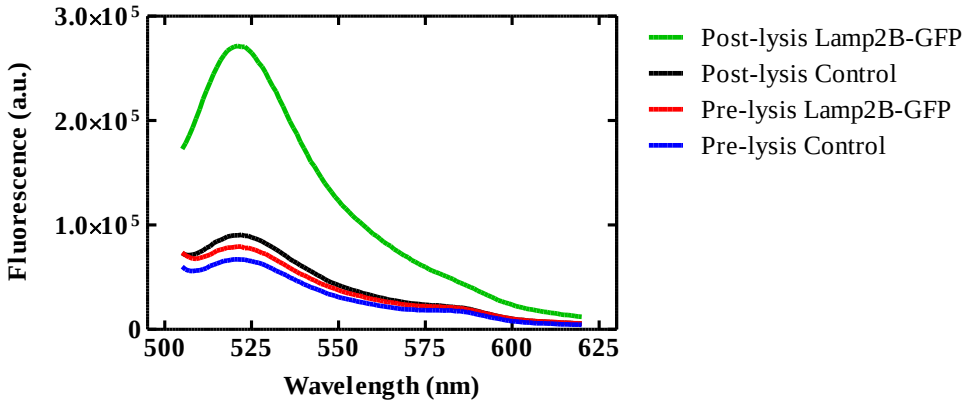
- Exosomes isolated and fluorescence characterized.
- Post-Ultracentrifugation the exosome pellet (containing either the lamp2B-GFP fusion protein, or control lacking this construct) was resuspended in 100 µL PBS. A 10 µL aliquot from each suspension was diluted with 240 µL PBS and examined by fluorescence spectrophotometry (using a Quanta Master 60 fluorescence spectrometer (Photon Technology International))
- *Fluorimeter settings:*

Excitation: 489 nm
Emission1: 505 nm -> 620 nm
Emission 2: 505 nm -> 620 nm

Slit Settings
Ex: Entr: 6 nm, Ctr: 40 nm, Exit: 6 nm
Em1: Entr: 12 nm, Exit: 12 nm
Em2: Entr: 12 nm, Exit: 12 nm

Step Size: 1 nm
Integration: 1 sec

- Following measurement of resuspended exosomes, exosomes were lysed by mixing 125 µL of each 250 µL exosome suspension with 125 µL of 1% sodium deoxycholate in PBS. Samples were incubated for ten minutes at room temperature prior to measurement. Fluorimeter settings were the same for measuring samples post exosome lysis as they were for measuring in-tact exosomes.
- Results:
- Data was plotted using GraphPad Prism v. 5.0



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Thursday, 11th

- Exosome samples characterized using confocal microscope and electron microscope

Monday, 15th

- RTH for s32-B0034-Uni started again. PCR to add RFC25 prefix and suffix to P1010 ccdB.

Tuesday, 16th

- RTH transformed . K105007 rehydrated and transformed

Wednesday, 17th

- Transformations grew, picked and grown overnight

Thursday, 18th

- Miniprepped RTH and send for sequencing.
- Miniprepped K105007 and started PCR to add NheI and PacI cut sites
- PCR to add NheI and PacI cut sites onto Lamp2b construct started

Friday, 19th

- K608351 and S32-RBS-Uni digested to begin assembly (K608351 upstream and S32-RBS-Uni downstream)
- Gel of digests ran and bands of interest gel extracted
- Extractions nandropped and ligated

Saturday, 20th

- * it looks like the replication origin on the lysis plasmids and the RNA-OUT plasmids are the same. This means they will both be competing for
- K808000-RNA-IN-K112808 and K808000-K112808 digested to be put into pSB2K3